EVALUATION OF RUMINAL EVACUATION VERSUS MARKER DILUTION PROCEDURES FOR ESTIMATING PARTICULATE PASSAGE RATE

by

ALISON ANN BEHARKA

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INTRODUCTION

The term passage (escape) is applied to movement of indigestible components through a compartment, usually the reticulo-rumen. More specifically, ruminal passage rate is the fraction of indigestible particles irreversibly exiting through the reticulo-omasal orifice per unit of time. Passage rate is expressed in units of 1/time or %/time. The reciprocal of passage rate is retention time, 1/(1/time). This is the length of time indigestible particles remain in a compartment. Additionally, rumen fill or volume is the mass of indigestible particles in a compartment.

In the forage consuming ruminant, the processes of intake, digestion, fill and rate of passage are highly interconnected, in that a change in one area can affect the other processes, and ultimately animal performance. Blaxter et al. (1956) noted that rations with the slowest particulate passage rates were digested to the greatest extent and the extent of digestion that can occur in the gastrointestinal tract is the direct combination of digestion rate and rate of passage. Additionally, rate of particulate passage has a theoretical relationship with intake (Grovum, 1983). Van Soest (1982) suggests that intake of poorer quality feeds is limited by reticuloruminal distension. Because digestion per se, is not believed to alleviate the volume a forage occupies in the reticulorumen, increased intake can only occur if rate of passage is increased. Therefore accurate estimates of the

nutritional status of the forage consuming ruminant depends on accurate estimates of intake, fill, rate of passage and digestion (Grovum, 1983).

LITERATURE REVIEW

Control Of Particulate Passage

Particulate passage is not an isolated function, there are many known and maybe some as yet unknown factors that influence and control passage rate (Warner, 1981).

Several theories exist regarding the rate-limiting processes of particulate passage. Grovum (1983) suggested that passage is limited by the rate of breakdown of large particles. Supporting evidence for this claim includes the increased rate of passage often observed with feeding ground and chopped roughages. In the ruminant, large feed particles may be somewhat reduced in size after ingestion by ruminal contractions and microbial degradation. However, the major reduction occurs by chewing during ingestion and rumination (Welch and Smith, 1970).

Small particles are generally believed to pass out of the rumen faster than large particles. Balch and Campling (1965) and Welch and Smith (1978) showed that fine particles are retained for less time than large hay particles, with the finer particles appearing to pass through the reticulo-omasal orifice more rapidly than the large particles. This was especially true in the first few hours after ingestion. Although Warner (1981) noted that some researchers found no effect of particle size on rate of passage, the majority of data suggests that passage through the reticulo-omasal orifice can only occur when particles have been reduced to a threshold

size. Reid et al. (1977) noted that particles less than 1mm in size were able to pass through the reticulo-omasal orifice without a further size reduction. The threshold size of 1.2 mm for sheep has been suggested (Martz and Belyea, 1986; Ulyatt et al., 1986) and a threshold size of 2.4 mm has been suggested for cattle (Ulyatt et al., 1986). Particle size discrimination probably occurs before the hindgut (Troelsen and Campbell, 1968; Ulyatt et al., 1986). Ehle and Stern (1986) noted that a 4x difference in particle size at dosing had no effect on particulate passage through the hindgut. Additionally, Fadalla et al. (1987) noted that abomasal particle size was not affected by the hay's original particle size.

Because 60-70% of the particles in the rumen at any given time are below the critical size for passage, and endoscopic observations of digesta movement through the reticulo-omasal orifice indicated that large particles (10mm) could clearly pass through (Mcbride et al., 1984), other factors besides particle size reduction must influence particulate passage. The rate of small particle exit has been suggested (Poppi et al., 1980;) to influence particulate passage. Mcbride et al. (1984) proposed that the sorting and physical stratification of large particles may influence small particle exit by entrapping them in the large particle raft. However, Welch (1982) noted that in some cases entrapment did not significantly delay passage. Therefore, rate of large

particle breakdown may influence exit of small particles by affecting the degree of physical stratification of rumen digesta. Regardless of which process actually is the rate limiting factor of particle exit, there are a number of factors that are known to exert general influence upon particle passage. These factors can be divided into dietary, animal, environmental and management factors.

Dietary Factors

The dietary factors that influence rate of passage include: 1) specific gravity of the feed particle; 2) forage to concentrate ratio; 3) cell wall content; 4) supplementation.

The specific gravity of a given feed particle may influence it's rate of passage through the digestive tract. Specific gravity is the ratio of mass or volume to an equal volume of water at 40^{o} C. Chemical composition is one of the major factors that can affect a forage's specific gravity (Martz and Belyea, 1986). One of the principle influences of chemical composition is on hydration potential. The more hydrated a forage is, the higher it's specific gravity. Carboxyl groups contained in hemicellulose and phenolic groups of lignin contribute substantially to ion exchange capacity and hydration potential, whereas cellulose resists hydration. When forage fiber is ingested, it has a specific gravity less than 1.0. As the particle is broken down, internal space is

decreased, hydration increases and releases entrapped air and rumen gases. Additionally the particle becomes more compact and particle density increases (Martz and Belyea, 1986). This increased density was demonstrated by Nocek and Kohn (1987) who employed an in situ technique to characterize changes in functional specific gravity over time. They reported that mean functional specific gravity increased with time in the rumen and that microbial fermentation and ruminal movement caused considerable increases in organic matter functional specific gravity. Increases in specific gravity have been suggested to increase the probability for escape because the heavy particles have been shown to shift through rumen stratification to a greater degree and become more accessible for passage than lighter particles (Durkwa and Welch, 1983). However, no single specific gravity or density value has been determined to be critical for passage. The fastest passage in cattle appears to occur at specific gravities between 1.1-1.2 (Campling et al., 1962). In addition, passage rates in the bovine rumen where shown to increase with specific gravities of 1.02 to 1.4, when compared to particles with a lower specific gravity; however, passage rate in the hindgut declined, indicating the effects of specific gravity may vary with compartment (Campling et al., 1962; Warner, 1981; Ehle and Stern, 1986).

Specific gravity and particle size may operate independently; however, research in this area is not

consistent. Ehle and Stern (1986) pulse dosed plastic spheres of specific sizes and densities and determined that both particle size and density influenced marker recovery and passage estimates. However, the response was greater for density than particle size, and there was no interaction between these two factors. This is contrary to the study by Durkwa and Welch (1982) who concluded that the influence of specific gravity (SG) on particle passage depended on particle size. They concluded from their study that short (1-5mm), light (SG=1.17) particles had greater recovery compared with long (1 cm), light (SG=1.17) and short (1mm), heavy (SG=1.77) particles.

Quality and type of diet have been observed to influence rate of particulate passage (Blaxter et al., 1961; Bines and Davey, 1970). Typically, rations high in cell wall content will have decreased passage rates (Ingalls et al., 1966; Wheeler et al., 1979; Prigge et al., 1984). Additionally, stems from a given forage have slower passage rates than leaves (Poppi et al., 1980ab). Cell wall content of forages is positively associated with time spent ruminating and negatively with intake (Welch and Smith, 1970).

The forage to concentrate ratio in the diet has also been shown to affect particulate passage (Eng et al., 1964), with passage rates usually decreasing as diet concentrate level increases (Goetsch and Galyean, 1983; Miller and Muntifering, 1984). Campling et al. (1962) showed that plastic particles

of specific gravity 1.12 had a mean retention time of about 60 h when fed to cattle on a hay diet. The mean retention time of the plastics increased to 110 h when fed with a high concentrate diet. Similarly, increasing the percentage roughage in the diet while holding feed intake constant will typically result in faster particulate passage rates (Evans, 1981; Grubb and Dehority, 1975). Bines and Davey (1970) increased the percentage of dietary forage from 20 to 40%, and from 40 to 60% of the diet, and increased passage rates in both cases. Increased salivation and rumination may occur as dietary roughage level increases, which may partially account for the faster passage rates (Harrison et al., 1976). In contrast, rations with very high roughage levels or roughages of poor quality will probably not show an increased rate of passage with a further increase in the percentage roughage in the diet. In fact, these diets often show an increased rate of passage in response to supplementation. This was illustrated by the increased rate of passage reported by Horn et al. (1984) who supplemented steers grazing wheat forage with silage and by McCollum and Galyean (1985) who supplemented steers fed poor quality prairie hay with a cottonseed meal supplement.

Protein supplementation often enhances voluntary intake of low quality forages (Bellido et al., 1981; McCollum and Galyean, 1985), and subsequently alters passage rate and(or) digestion. Steers supplemented with cottonseed meal increased

their intake of prairie hay and demonstrated increased particulate passage rates (McCollum and Galyean, 1985). However, supplemented sheep exhibited increased intake, but no subsequent change in particulate passage rate (Krysl et al., 1986). The effect of supplemental protein on passage rate varies (Caton et al., 1986ab; Judkins et al., 1987; Branine et al., 1986). Branine et al., (1985) speculated that the forage N content has an effect on response to supplemental protein.

Addition of buffers (Okeke et al., 1984), NaOH (Zorrila-Rios et al., 1985) and ammoniation (Berger et al., 1980) may increase passage rates. Supplementation with urea alone or combined with carbohydrates, usually molasses, has yielded variable responses (Johnson, 1976). Ortigues et al. (1988) supplemented wethers fed poor quality fescue hay with various combinations of urea, molasses and corn and reported no effect on passage rate when compared to controls. This agrees with Redman et al. (1980), but disagrees with other researchers who have shown increased rate of passage estimates with urea supplementation (Campling et al., 1962).

Supplementation with products such as amino acids, that furnish growth factors for the ruminal microbial population, is a relatively new area and may potentially influence particulate passage rates (Clark and Peterson, 1988). Cows fed a supplement designed to supply 250 g crude protein exhibited increased particle passage and fermentation rates when DL-

methionine, a limiting amino acid for bacteria (Salter et al., 1979), was included in the supplement (Clark and Peterson, 1988).

Animal Factors

The animal factors that can influence particle passage include: 1) dry matter intake; 2) rumen motility; 3) animal species; 4) physiological status and 5) individual animal variability.

There are numerous reports of increased feed intake associated with increased rate of passage in both sheep, and cattle when the same diets were evaluated at graded levels of intake (Campling et al., 1962; Minson, 1966; Grovum and Williams, 1977; Colucci et al., 1984). Balch and Campling (1965) suggested that the increased intake resulted in an increased passage rate as a result of the increasing distension in the reticulorumen. However, when a diet change accompanies the increase in intake, rate of passage does not always increase (Waldo et al., 1965; McAllan and Smith, 1976), because rate of passage values of a dietary component are influenced by all the components of the diet.

Uyatt et al. (1986) demonstrated that organic matter flow in sheep fed lucerne hay was lower per reticular contraction for low intake sheep, than high intake sheep. Therefore, they proposed that the amount of material passed per reticular contraction affects passage. Similarly, several researchers have indicated that ruminal motility is important in the

movement of ingested, marked meals out of the reticulrumen (Ehrlein, 1979; Desweyen and Ehrlein, 1981). McBride et al. (1984) proposed a sequence of digesta transfer from the reticulorumen. In general, the reticulum contracts forcing its contents over the reticulo-ruminal fold into the ventral rumen and cranial sac. The reticulo-omasal orifice opens and the digesta is either moved through by contractions or into the reticular groove.

Animal species has been shown to influence rate of particulate passage (Warner, 1981). In general, cattle have slower passage rates than sheep. Prigge et al. (1984) noted digestibility differences between steers and wethers of six percentage units when fed ad libitum, but only one unit difference when fed at a restricted level. Solid retention times in this experiment were 50% greater for cattle than sheep. Since there was no species differences observed for in situ dry matter disappearance over time, the authors concluded that the observed difference in dry matter digestibility was due to differing passage rates, not to differing rates of digestion. This agrees with Poppi et al. (1980) who indicated that species differences were due to differences in particle passage rate.

Passage rate like most other digestion functions varies tremendously between animals even of the same breed and species. Warner (1981) proposed that animal age, exercise and physiological status may influence passage of particulate

matter. Particulate passage rate has been shown to increase with advancing pregnancy in cattle (Graham and Williams, 1962) and sheep (Forbes, 1970). However, Putman et al. (1967) found no passage rate differences for cattle at five months compared with seven months pregnant. Further research is needed in this area. Once the female gives birth, particulate passage increases significantly (Pond et al., 1984), with stage of lactation possibly influencing passage rate.

Differences in particulate passage rate have been reported for animals of the same breed, sex and physiological status. Purser and Moir (1966) reported that sheep whose weights ranged from 61.8 to 75.5 kg had ruminal volumes that varied between 2.5 to 7.6 liters. They suggested that individual differences in rumen volume led to differences in consumption of poor quality roughage, digesta present in the rumen and rate of particulate passage.

Management And Environmental Factors

Management and environmental factors that influence particle passage include: 1) feeding frequency (FF); 2) grinding and pelleting; 3) climatic variables. Frequency of feeding is often mentioned as a possible influence of particulate passage, however little specific work has been done in this area. Coleman et al. (1984) conducted a series of experiments to determine the effect of feeding frequency and markers on particulate passage estimates per se. Using steers dosed with Yb-labeled feed and chromium, they reported

that particulate passage rate was significantly influenced by a FF x marker interaction. Similar turnover rates were observed with Yb and Cr when steers were fed twice daily (Yb=2.8 and Cr=2.6 %/h) but rates were different when steers were continuously fed (Yb=2.0 and Cr=1.5 %/h). These results may have been slightly confounded because intake was slightly higher for the 2x versus continuously fed steers.

Feeding regimens in which large meals are offered once or twice daily result in diurnal fluctuations in ruminal fermentation that may detract from forage fiber digestion and microbial protein synthesis (Johnson, 1976). Subsequently, Bunting et al. (1987) suggested that maintenance of a stable and efficient ruminal microflora requires continual and adequate input of feed during the day. These researchers noted that as FF went from 2 to 16x daily for lambs fed tall fescue hay, water consumption increased 35% (Bunting et al., 1987), which agrees with Ulyatt et al. (1984), who also reported 35% increases in water consumption when FF was increased from 1 to 24x. This increase in water consumption may cause an increased rate of ingesta removal from the rumen (Ulyatt et al., 1984) possibly resulting in greater escape of potentially degradable substances.

Feed particles whose size has been mechanically reduced by grinding, chopping or pelleting exhibit increased rate of passage when compared to coarse hay (Balch, 1950; Blaxter et al., 1956; Alwash and Thomas, 1971). Faichney (1983) reported

that as hay intake in sheep increased towards ad libitum levels, passage rate increased more rapidly when the hay was ground and pelleted than when it was simply chopped. An explanation might be that ground diets let larger particles pass out more frequently because there is less fiber mat to catch them (Pearce and Moir, 1964).

Changes in environmental temperature may affect particulate passage rates, with cold temperatures seeming to increase passage rates (Westra and Christopherson, 1976; Kennedy et al., 1977). Similarly, Warren et al. (1974) reported that cattle held at 32^{o} C had a slower passage rate than those held at 18^{o} C. Kennedy et al. (1977) noted that the largest difference in passage rate occurred in the rumen, as there was no significant effect of temperature on the rate of passage of marker through the hindgut. In addition to the increased rate of passage, reticular motility in sheep has also been reported to increase as temperature dropped from 21.1 to 1.3^{o}C (Westra and Christopherson, 1976). The mechanism resulting in increased motility in the cold has not been determined, but might involve an alteration in the activity of the gastric centers in the medulla oblongata.

Techniques For Measuring Rate Of Particulate Passage And Fill

There are two general techniques used for estimating particle passage rate: 1) direct measurement; 2) measurement via marker-based procedures. Because both methods have

shortcomings, choice of a method depends on the experimental situation and objectives.

A wide variety of markers have been used to study digestion kinetics in the ruminant. Markers are especially used in situations where use of direct methods are inconvenient or impossible (e.g., intact animals). The characteristics of these markers have been extensively reviewed (Kotb and Luckey, 1972; Englehardt, 1974; Faichney, 1975). These authors have summarized the criteria of the ideal marker : 1) inert, not metabolizable or absorbed; 2) it must not affect or be affected by the gastrointestinal tract or its microbial population; 3) assay must be precise and specific; 4) it must be physically similar to or intimately associated with the material it is to mark. Englehardt (1974) noted that no marker in use today can completely satisfy all these criteria, but several are considered to be useful.

Markers can be divided into three groups, liquid, microbial and particulate, based on the digesta component they are associated with. The particulate markers can be divided into two groups based on their origin. Internal markers are naturally occurring, indigestible components of the feed. External markers are not normal components of the feed, they are fed or otherwise administered to the animals (Pond et al., 1987).

Particulate markers

Stained feed particles probably were the first

particulate bound markers used (Kotb and Luckey, 1972). In 1970, Asplund and Harris concluded that because no satisfactory method has been found to quantitatively measure the recovery rate of stained particles, the use of this method is limited. However this method was widely used during the 50's and 60's (Warner, 1981). A recent study by Fadalla et al. (1987) investigated the suitability of two of the more commonly used dyes for staining, brilliant green and basic fuschsin, as particulate markers. They reported that brilliant green and basic fuschsin resulted in large and moderate depressions, respectively, of oat husk digestibility. Additionally, particulate passage rate for the basic fuschsin was 40% slower than for a rare earth marker.

Uden et al. (1980) proposed a mordanting procedure in which chromium is tightly bound to the feed particle, rendering it indigestible and inseparable during digestion. This Cr-mordanted fiber can be used as a particulate marker. This procedure has the advantage of a very strong binding between the chromium and the plant cell wall. However, because this procedure significantly reduces fiber digestibility and increases its density, flow rates associated with this marker can be misleading (Ellis et al., 1980; Ehle et al., 1984). This problem may be minimized if chromium is added at a lower percentage of the fiber weight than has commonly been used (Pond et al., 1987). Because the mordanting procedure is only associated with chromium, the procedure is not suitable for

situations were multiple marking is desired (Ellis et al., 1980).

Rare Earths

The rare earths are a family of elements that have similar properties (Ellis, 1968; Lucky et al., 1977) and have gained considerable attention in recent years as external particulate bound markers (Ellis, 1968; Ellis et al., 1979; Teeter et al., 1979; Ellis et al., 1980; Teeter et al., 1984). A particular advantage of using rare-earth markers is that multiple markers may be used simultaneously while studying particle digestion and passage (Pond et al., 1985). Elements that have been investigated include cerium (Ellis and Huston, 1968; Huston and Ellis, 1968), dysprosium (Ellis, 1968), ytterbium (Ellis et al., 1979; Teeter et al., 1984), rubidium, lutetium, lanthanum, erbium, and terbium (Pond et al., 1987). The most commonly used rare earth is ytterbium. Ytterbium has an advantage over the lighter rare earths such as samarium, lanthanum and cerium in that it has a higher binding affinity for feed components (Sinha, 1966). Therefore, ytterbium exhibits less migration to other feed particles during the digestive process (Lascano and Ellis, 1979; Teeter et al., 1979).

Rare earths were initially proposed as particulate markers based on observations that radioisotopes of these elements from atmospheric fallout were almost completely recovered on plant foliage (Morgan, 1959), indicating a high

binding capacity. Later work demonstrated that because the rare earths posses adsorptive properties they can be used to label particulate matter directly.

The specific nature of binding between rare earths and feed residues in ruminal digesta has not been extensively investigated. However, it has been suggested that covalent bonds occur between the rare earths (the electron acceptor) and various ligands of the feedstuffs (the electron donor; Ellis et al., 1980). The functional groups that are involved in solute-binding of Yb are not known; however, possibilities include carboxyl and hydroxyl groups as well as amino acid nitrogen (Teeter et al., 1984). It should be remembered that feedstuffs are intact tissues, therefore surface components such as epidermal tissues and fractional cell walls are the primary cellular structures exposed to Yb. Intracellular components such as protein and nucleic acids may also be able to bind Yb, but the opportunity to do so is limited. Ellis and Beever (1985) speculated that the binding forces were heterogenous in nature and result in many different binding affinities. They also noted that each individual binding site could be described by an association constant (ka). Teeter et al. (1984) measured the binding affinity of various feeds and Yb. They concluded that binding affinities for Yb varied with feedstuff, indicating that: 1) the functional groups that bind Yb vary with the feedstuff; 2) the molecular environment of functional groups varies with the feedstuff; 3) some types of

particulate matter form multiple bonds with Yb (Teeter et al., 1984).

A number of studies have been conducted to test the tenacity with which rare earths remain bound to feed particles and their possible migration to previously unbound feed residues (Lascano and Ellis, 1979). Degree of migration appears to depend on the feedstuff labeled and the labeling technique, with forages exhibiting less migration than grains. Lascano and Ellis (1979) bound Yb to the cell wall constituents of sieved, masticated hay and measured Yb content of various sizes of fecal particles. Since the Yb concentration was similar across all fecal particle sizes (1600 to < 60 um), the authors concluded that little marker migration had occurred.

Erdman and Smith (1985) bound Yb to the cell wall fractions of different forages and then sized fractions within each forage via sieving, to determine the relative binding of Yb. The alfalfa hay exhibited better recovery than the corn silage. A possible explanation is that binding affinities are higher for feeds with high fiber and protein contents (Teeter et al., 1984). As sieve size increased, Yb recovery was reduced. The authors suggested this indicates that the smaller particles had greater surface area per unit mass allowing for more potential binding sites. There was also a forage x particle size interaction. The authors concluded that labeling of the entire forage would bias the passage measurement.

Hartnell and Satter (1979) measured the movement of the rare earths Sm, La and Ce from labeled hay and grain to unmarked feedstuffs. They determined movement to be less than 1% from labeled hay but up to 7% from the labeled grain. This agrees with Combs et al. (1984a,b) and Turnball and Thomas (1987) who used a 2 stage in vitro fermentation in which Yb marked corn was incubated with Coastal bermudagrass hay. When the fermentation study was terminated at the end of the fermentation phase 90% of Yb was recovered, of this, 8.8% had migrated to the hay and 2% was recovered in the supernate.

Even though the rare earth/feed complex is reasonably stable in the rumen, (Hartnell and Satter, 1979; Smith et al., 1983), migration of rare earth from a labeled feed particle to an unlabeled feed particle may be expected if the quantity of rare earth applied to the feed particle exceeds the particle's binding capacity (Ellis et al., 1981). The stronger binding sites of most feedstuffs have limited binding capacities for rare earths, ranging from .2 mg of Yb/g whole corn to 9.7 mg of Yb/g soybean meal (Ellis et al., 1981). Other reasons for marker migration are: 1) disequilibrium between particulate-bound rare earths and the liquid phase; 2) not removing easily exchangeable rare earth before dosing; 3) exposure to feed particles with higher association constants for the rare earth (Teeter, 1979; Teeter et al., 1984). Combs et al. (1984a) reported that up to 37% of the rare earth metal dosed was recovered in the bacteria following

incubation during in vitro ruminal fermentation studies. Additionally, in the acidic environment of the abomasum, the increasing H+ concentration can competitively displace the rare earth markers (Ellis et al, 1983). This is further illustrated by Turnball and Thomas (1987) who reported that after the acid-pepsin phase of an in vitro fermentation trial, only 7.9% of the Yb-marker was recovered on corn and 36% on hay. In contrast 57.4% of the Yb-marker was recovered in the acid pepsin supernate. Ellis et al. (1983) noted there are binding sites which resist dissociation at a pH as low as 1.5 and therefore it may be possible to selectively bind Yb to these sites.

Some marker procedures such as mordanting fiber render the feed particles less digestible. Mader (1980) evaluated the effect of Yb binding upon the digestibility of several feeds. In vitro digestibility of wheat forage was lowered by 15, 21, and 34% for early, middle and late maturity wheat forage, respectively, by saturating the binding sites with Yb. Prairie hay in vitro DM digestibility decreased by 9, 14, 20, and 27% as the quantity of Yb bound increased from 10000 to 32000 ppm. These results suggest that particles in the vicinity of the bound metal may exhibit reduced digestibility. Mader et al. (1984) reported a decrease in DM digestibility using a 2-stage in vitro ruminal fermentation when sudan grass was marked with rare earth markers. Teeter et al. (1984) suggested that this reduced digestibility would have little effect on

passage rate determinations unless the particles were not small enough to leave the rumen. Teeter et al. (1984) reported that marker application to grain and forages decreased in situ DM digestibility. A recent study by Smith et al. (1987) indicated that treatment of wheat forage cell walls with Yb affected the rate of marker disappearance from a continuously fed fermenter. They suggested that caution was needed in interpretation of results from different experiments.

Marker Methodology

Many methods have been used to label particulate matter with rare earths. The rare earth solution can be sprayed directly onto the material being prepared for dosing (Ellis and Huston, 1968; Olbrich et al., 1971; Hartnell and Satter., 1979). When labeling is accomplished by sprinkling or spraying, however,local concentration of marker may exceed the binding capacity of the feedstuff (Pond, 1982). These problems can be reduced by soaking the material to be labeled in the rare earth solution followed by rinsing to remove the unbound rare earth (Teeter et al., 1979). This method had the advantage of removing any excess or loosely bound Yb before dosing and preventing loss of Yb in the fluid phase of the digesta. Pond (1982) determined that rare earths applied by the soak-and-rinse procedure can be utilized as particulate flow markers in ruminants with similar results as obtained when using the Cr-mordant procedure. Ellis and Beever (1985)

recommended two binding procedures based on the assumption that it is possible to bind rare earths to feed particles in a fashion that minimizes proton-induced dissociation in the abomasum. The first procedure involved soaking the rare earth-feedstuff complex in acetate to achieve high levels of rare earth binding to the higher affinity binding sites. The second procedure used EDTA in place of the acetate to allow binding at pH 7. The correct binding technique to use varies with the feedstuff used and the experimental objectives (Teeter et al., 1984).

Additional research is needed to determine the best material to label. Theoretically, if the marker does not migrate anything can be labeled. In practice, however, labeling of a less-digestible feed component (e.g., NDF residue, fecal matter) may provide a more accurate estimate because rare earth markers have been shown to migrate under several conditions.

The rare earths can be administered either in a single "pulse" dose or by "continuous" infusion. These methods would be followed by sampling at successive times or, for continuous infusion, killing the animal and sampling from various gastrointestinal sites. With the continuous infusion technique, the marker can be administered daily in the diet or infused directly into a compartment. When equilibrium is achieved, flow rates from each sampling point can be calculated once the concentration of marker in the compartment

is determined. After marker infusion is stopped, samples can be taken over time to determine compartment volume and mean retention time. A pulse dose followed by time-sequence sampling will also yield mean retention times, volume and flow rates (Faichney, 1975; Ellis et al., 1980). Pond (1982) investigated the influence of time of dosing, relative to meal consumption on passage rate estimates. It was concluded that since passage rates were 42% higher when capsules were dosed at the beginning of a meal compared to the end of the meal, dosing before a meal allowed for better mixing.

Administration of the marker can be accomplished several ways. Gelatin capsules containing water soluble rare earth or marked particulate matter can be administered orally or directly into the rumen (Miller and Byrne, 1970ab; Miller et al., 1971). Labeled particulate matter can be fed or administered via the cannula with the material being placed either on top of the hay mat or stratified throughout the middorsal to midventral region of the rumen. Cochran et al. (1986a) reported that there was no significant difference in passage rate estimates derived by the various dosing strategies for steers fed prairie hay.

When dosing rare earth markers, the size of the initial dose is limited by the binding capacity of the particular feedstuff. Teeter et al. (1984) noted that the amount dosed should supply 200 ppm Yb in the digesta, and the Yb applied to the material to be dosed should be sufficient to saturate

50-120% of the material's total binding capacity. For forages and protein complexes, 50% saturation will be sufficient to achieve proper binding levels because of the high binding capacity these feeds possess. Grains require a higher saturation level and a longer immersion time to ensure adequate binding. To avoid precipitation of the hydroxide form of the rare earth, feedstuffs should be labeled as soon as possible after the solution is prepared. If storage is necessary, the pH should be maintained below four (Ellis et al., 1980).

There is still insufficient evidence to outline a specific sampling schedule. Samples can be taken from any cannulated compartment or the rectum, however site of sample may affect passage estimates. Coleman et al., (1984) reported that steers dosed with Yb had significant differences in passage rate between rectal and ruminal sampling. They speculated that ruminal sampling may be more sensitive to changes in intake. However, Hartnell and Satter (1979) suggested that fecal grab samples may be more desirable due to mixing and sampling problems associated with ruminal samples. Samples taken in the rumen should be from several different sites; however, mixing ruminal contents before subsampling does not appear necessary (Cochran et al., 1986a). The sampling regimen depends on the site and the model used to determine rate of passage. A general guideline is to collect a 0h sample, administer the marker, then collect

samples at 4, 8, 12, 16, 24, 32, 48 h post dosing for ruminally derived rates and 4, 8, 12, 16, 20, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96, 108, 120 h postdosing for fecally derived rates. Samples should be 200 to 300 g fresh weight. Fecal samples can be easily obtained by rubbing the top of the rectal wall.

Analytical Procedures

There are several different procedures that can be used to determine the rare earth content of a sample. Choice of procedure depends on equipment availability, time, cost, and sensitivity of detection needed. Automated multichannel counting of Gamma emitting radioisotopes offers high sensitivity for detection and a quick analysis (Ellis et al., 1980). Radioactivation analysis can also be used if the facilities are available (Ellis, 1968), however the cost can be relatively high and requirements associated with handling radioisotopes are extensive and can be quite restrictive. Plasma optical emission spectroscopy may yield a fairly sensitive detection but more research is needed on this procedure (Fassel and Knisely, 1974). Neutron activation analysis may be the most quantitative analytical technique and also the method that can detect the rare earths with the most sensitivity (Pond et al., 1985). Minimum detectable levels within a digesta matrix in a 1 g sample (in ug) are; Yb .07; La .06; and Sc .004 (Pond, 1982). Neutron activation analysis also allows for the simultaneous detection of many

markers and metals in a single sample. However, equipment needed is not universally available and the technique can be expensive.

Probably the easiest and least expensive method of analysis is by atomic absorption spectrophotometry using a nitrous oxide flame. This method provides marginally sufficient sensitivity for Yb, Er and Dy. There are other extraction procedures that can increase detection sensitivity. Procedures that utilize strong chelating agents such as EDTA and DPTA at neutral or slightly alkaline pH will reduce the level of undesirable salts that can alter rare earth absorption during analysis. These agents can extract the rare earths from most organic and ash samples (Ellis et al., 1980). A procedure utilizing .05 M EDTA extraction is that suggested by Hart and Polan (1984). These authors suggest placing the sample in 25 ml of EDTA solution, shaking it for 30 min and then filtering through Whatman #4 filter paper. Lascano and Ellis (1979) demonstrated that refluxing a sample for 2 hrs with a 0.1 M EDTA at pH 7 removed 50 - 80% of the rare earth applied. Ytterbium can also be extracted from dry ashed feed, digesta and fecal samples using 3 M HNO_3 and 3 M HCL (Ellis et al., 1980).

Mathematical Models

Rate of passage can be estimated by using an exponential equation if steady state conditions occur. This assumption

will allow for a constant passage rate and an implied exponential lifetime of particles in the compartment of interest (Ellis et al., 1980).

The simplest model based on these assumptions is the exponential decay model. Mathematically this model can be expressed as:

$$C_{e(t)} = C_o e^{-kt}$$

Were, $C_{e(t)}$ = concentration of marker on particles emerging or disappearing from a compartment at sampling time "t".

C_o = concentration of marker in compartment the instant after dosing.

k= fractional rate constant

This model is usually used when dosing and sampling are done in the same compartment (e.g., rumen). The volume of particles comprising the dilution pool of particles can be calculated from the dose of marker (D) (Ellis et al., 1980 and Pond et al., 1987).

$$\text{Fill (g)} = \frac{\text{Marker administered (ug)}}{C_o \text{ (ug/g)}}$$

Interest in describing passage rates in intact animals led to attempts to mathematically fit fecal marker excretion curves.

When researchers were examining the fecal excretion curves, they noted that the marker appeared in the feces as if it went through more than one compartment (Ellis et al., 1980). Several models were subsequently proposed to account for more than one compartment in the gastrointestinal tract (Blaxter et al., 1956; Hungate, 1966) Grovum and Williams (1973) proposed a 2- compartment, time-independent model which assumed the existence of two compartments and an exponential lifetime distribution for particles in each of the compartments. Mathematically this model can be expressed as:

$$C_{e(t)} = C_o e^{-k1(t-TT)} - C_o e^{-k2(t-TT)}$$

were, TT= transit time of marker through the intestine

k_1 = slow rate = fractional rate constant representing passage from rumen.

k_2 = fast rate = k2 = fractional rate constant representing passage from the cecum/proximal colon.

While k_1 provides an accurate estimate of ruminal marker decay, k_2 is probably a confounded estimate (Grovum, 1983).

Matis (1972) was the first to suggest that the lifetime distribution of particles may not be exponential, based on the reasoning that newly introduced particles don't instantly mix

with other particles previously in the compartment. Further, newly ingested larger particles require reduction in size to increase their probability of exit. Thus, newly ingested large particles have a very low probability for exit as compared to older, smaller particles. Since these processes require time, a portion of the factors determining passage are related to time of residence in a compartment. Gamma lifetime distributions, mainly G_2, have been proposed to represent age dependency. Subsequently, gamma time dependency has been incorporated in a one (1cmpt) and two (2cmpt) compartment model (Ellis et al., 1980) to estimate passage rates.

The advantage of using a more complex mathematical model is still in doubt. Cochran et al. (1986a) using steers fed ad libitum quantities of prairie hay found minor differences among models, however differences did not influence interpretation of the treatment effects. Coleman et al. (1984) used Yb as a particulate marker and concluded that inherent variation in sampling and marker analysis may preclude the use of complex models containing two or more pools, especially with forages. When time-dependent models have been applied to grazing studies, the one compartment model with time delay and gamma two age dependency has given the best fit (Pond et al., 1987). However, different animals and different treatments may require different models to get adequate estimation of parameters (Pond et al., 1987).

Internal Markers

An alternative method for determining particle passage is the use of direct measurement. Rate of passage can be easily calculated, by recording the animal's daily dry matter intake and completely removing the reticuloruminal contents, followed by weighing and subsampling the contents (Reid, 1965). An internal marker is then isolated from subsamples of feed and digesta, and is used to form a ratio of intake to mass:

$$Kp = \text{rate of passage } (1/h) = \frac{\text{intake of indigestible particles (kg/h)}}{\text{mass of indigestible particles in rumen (kg)}}$$

This method is sometimes criticized due to the need for cannulated or sacrificed animals and for methodology problems, such as when to evacuate and which internal marker to use. However, this method has some advantages compared with marker based procedures. First, this procedure allows a direct measurement of ruminal fill. Secondly, direct measurement avoids problems associated with marker systems such as marker migration and choice of mathematical model. Thirdly, it is a less time consuming procedure that requires fewer samples to prepare and analyze.

Early fears about disrupting anaerobic conditions by ruminal evacuation appear to be unfounded. Towne et al. (1986) demonstrated that ruminal evacuation had no effect on the

microbial population, volatile fatty acid concentration and liquid flow rate of evacuated steers. However, time of evacuation could have a significant effect on passage rate estimates and must be considered when planning the experiment.

A wide variety of feed components have been suggested as possible internal markers. Use of many of these markers has been limited due to data illustrating marker unsuitability. Others are still used in spite of their shortcomings. Internal indicators commonly used include lignin, silica, acid insoluble ash (AIA), indigestible acid detergent fiber (IADF) and indigestible neutral detergent fiber (INDF) (Kotb and Luckey, 1972; Penning and Johnson, 1983; Cochran et al., 1987).

Silica is probably one of the older internal markers, however, it also appears to be relatively unreliable (Kotb and Luckey, 1972). Silica recovery in feces of grazing animals and confined ruminants (Kotb and Luckey, 1972) was not quantitative. Silica may be absorbed and excreted through the urine. In addition, soil and dust contamination can increase the amount of silica excreted. Therefore, silica is not a useful internal marker in most cases. Although silica per se has questionable value as an internal marker, acid insoluble ash, a crude silica isolate has received considerable use over the past 20 years. Information regarding this procedure has been contradictory. While some researchers have reported that AIA is a reliable marker for predicting digestibility in

cattle (Thonney et al., 1985; Taniguchi et al., 1986) and sheep (Block et al., 1981), other researchers reported that AIA yielded digestion coefficients significantly different from those derived by more conventional means (Chase and Hibberd, 1985). Therefore, this procedure should be used with caution, especially under conditions were soil ingestion my take place.

Kotb and Luckey (1972) noted that as early as 1871 researchers felt that lignin was not digestible by the ruminant. However in 1928 incomplete recovery was already being reported (Kotb and Luckey, 1972). Early research utilizing lignin as an internal marker was severely hindered by lack of knowledge about lignin structure. This is still somewhat of a problem today, although to a lesser degree. The composition of lignin varies considerably among feeds and even among the individual feeds within a group (Reeves, 1985). This variation in lignin composition is not directly related to the total amount of lignin present (Reeves, 1985). Patterns in lignin composition do emerge when the lignin content of forages is examined. They are: 1) as physiological maturity of a plant advances lignin content increases (Morrison 1980; Allinson and Osbourn 1970); 2) legumes generally have a higher lignin content than grasses (Allinson and Osbourn, 1970), however, legume lignin is less soluble in alkali (Jung and Fahey, 1983); 3) grass lignins are not as condensed as legume lignins (Gordan, 1975). It should be noted that the monomer

units comprising lignins vary with forage species, therefore, lignin is not a single substance, but a class of substances.

Because there is not a good analytical procedure to determine true lignin, the actual quantitative and qualitative nature of lignin is still not precisely known (Van Soest, 1982). Lignin analysis is made more difficult because not all of the polymerization of lignin is the result of enzymatic action. An almost random series of bonding occurs giving lignin a very complex structure which is not susceptible to simple hydrolysis (Jung and Fahey, 1983). By exposing plants to labeled compounds it has been determined that true lignin is a polymerized product of phenylpropanoid alcohols, ferulic and para-coumaric acids (Van Soest, 1982). The lignin complex has been theoretically divided into two parts, the "core" lignin and the "noncore" lignin. Noncore lignin is probably made up of predominately p-coumaric and ferulic acid. These acids contain both hydroxyl and carboxyl functional groups, that can act as cross links between lignin and structural carbohydrates (Jung and Fahey, 1983). Core lignin refers to the highly condensed phenylpropanoid matrix which appears to be linked to the structural carbohydrates of the plant cell wall via carbohydrate esters of ferulic acid (Gordan and Neudoerflu, 1973).

True lignin is probably indigestible during gastrointestinal transit in the ruminant (Neilson and Richard, 1978). To support this claim researchers note that to date,

there has been no mammalian or bacterial enzyme isolated that can degrade polymerized phenols (Van Soest, 1982). Additionally, lignins, as well as phenolic monomers, inhibit microbial growth of both aerobes and anaerobes (Jung and Fahey, 1983). Exposure to phenolics apparently results in membrane damage, lysis of bacteria and release of cell contents (Jung and Fahey, 1983). However, bacterial degradation of phenolic monomers bound to the cell wall and soluble lignin-carbohydrate complexes formed in the rumen have been reported (Jung and Fahey, 1983). It is doubtful that the negative fecal recoveries of lignin reported by some researchers was caused by bacterial degradation of lignin. Both positive and negative recoveries of fecal lignin, leading to possible negative and positive estimates of lignin digestibility have been reported (Fahey and Jung, 1983; Cochran et al., 1987). Positive recovery could occur if: 1) artifacts were formed during gastrointestinal transit; 2) artifacts were formed during analysis. Negative recovery could occur if: 1) lignin with a low degree of polymerization and low molecular weight was absorbed and excreted in the urine (especially true with young plants); 2) small lignin particles were lost during filtration; 3) the lignin recorded was contaminated with potentially digestible compounds.

Fahey and Jung (1983) noted that changes in the lignin complex can occur from the time it's ingested to the time it's passed out. Porter and Singelton (1971) used sheep with re-

entrant duodenal fistulas fed hay and straw diets to determine that lignin was digested from 1.2 to 10%. When the lignin in the feed and feces was analyzed, no changes in the carbon, hydrogen or nitrogen content occurred, however methoxyl content in fecal lignin decreased 19.2% for hay and 33.5% for straw compared to feed lignin. They conducted further experiments consisting of measuring the quantity of lignin passing through the duodenum and found it similar to the quantity in the feces. They concluded that there was little or no lignin degraded in the intestine, Further research into the stomach as the site of lignin degradation was conducted by Gaillard and Richards (1975) using cattle fed grass. They demonstrated that soluble lignin-carbohydrate complexes were formed from lignin in the rumen. Neilson and Richards (1978) used in vitro experiments to conclude that the soluble lignin carbohydrate complex was not affected by further digestion in the rumen after passing into solution. The complex precipitated out in the abomasum and ended up in the solid matter in the feces. No evidence of true lignin digestion was shown in either experiment. The authors implied that a large amount of total dietary lignin may pass into solution in the rumen and therefore, this dissolved lignin might pass through the rumen at a faster rate.

In spite of all the before mentioned problems, most researchers believe that true lignin is indigestible. This assumption suggests that a procedure can eventually be found

that will recover true lignin. No procedure in widespread use today will recover a chemically distinct lignin (Van Soest, 1982). Lignin can be measured with direct gravimetric methods, or through indirect methods that measure lignin as loss in weight. Additionally, spectrophotometric methods have been proposed (Van Soest, 1982).

The ADF-Klason procedure is the direct method that gives the lowest lignin value. It is based on the original Klason method designed for wood (Van Soest, 1982). Problems associated with this procedure include recovery of Maillard polymers and cutin, and removal of some ferulic acid and true lignin (Van Soest, 1982). Indirect methods include the use of triethylene glycol-HCL and the ADF-permanganate procedure (Van Soest, 1973). These methods may recover non-lignin carbohydrates such as pectin and therefore may result in a positive lignin recovery.

Spectrophotometric methods for determination of lignin are based on UV absorbance. Lignin is extracted by alkali and the absorbance of the filtrate is measured (Van Soest, 1982). The problems associated with this method are: 1) nonlignin matter can interfere with absorbance and 2) optical density is altered by oxidation of lignin in alkaline solutions. Unless improvements in this method are made, it should be considered an unsuitable method of lignin determination.

A potentially promising internal marker which incorporates some standard lignin methodology has been

suggested by Cochran et al. (1987) in which the internal marker is isolated by alkaline hydrogen peroxide incubation followed by acid detergent lignin extraction (alkaline peroxide lignin = APL). This method was based on the hypothesis that the lignin fraction resulting from alkaline hydrogen peroxide treatment may eliminate some types of artifact lignin and, therefore, have less recovery problems than previously mentioned lignin fractions. Cochran et al. (1987) reported APL recoveries with fresh immature bluestem grass of 99%, and noted that the APL treatment gave an OMD coefficient similar to OMD determined in vivo when cattle were fed dormant bluestem range. They concluded that APL appears to be a viable internal marker for both immature and dormant bluestem range diets. However, additional research is needed to determine the value of the APL procedure across a wider variety of diets (Cochran et al., 1988).

Indigestible acid detergent fiber (IADF) and indigestible neutral detergent fiber (INDF) are relatively new internal markers (Berger et al., 1979; Penning and Johnson, 1983). However, studies using fiber as internal markers were suggested in the 1950's (Kotb and Luckey, 1975). It was not until the 1960's when Van Soest et al. (1966) reported that particles of cell-wall origin made up the majority of residues in the ruminant feces that attention was focused in earnest upon the use of such residues as internal markers.

Neutral detergent fiber is defined as cell wall

constituents insoluble in neutral detergent solution and consists primarily of hemicellulose, cellulose, lignin, fiber bound protein and other lignified nitrogenous compounds (Van Soest, 1966). Acid detergent fiber represents the cell wall constituents insoluble in ADF solution and consists primarily of cellulose, lignin and lignified N. Indigestible NDF or ADF fiber is the NDF or ADF residue that remains after digestion by rumen microbes with or the without pepsin/ HCL step (Ellis et al., 1984).

Present day procedures for determining IADF and INDF are based on the original suggestion of Van Soest et al. (1966). This original procedure consisted of a 48-h in vitro fermentation, (Tilley and Terry, 1963) followed by neutral detergent extraction (Goering and Van Soest, 1970). Several modifications of the original procedure have been evaluated. Berger et al. (1979) extended the fermentation time from 48 to 96 h and used acid detergent in place of neutral detergent. Waller et al. (1980) included a 16 h acid pepsin treatment before the in vitro fermentation phase. Many current procedures consist of a 6-d in vitro fermentation followed by neutral or acid detergent extraction (Ellis et al., 1984; Lippke et al., 1986). Lippke et al. (1986) reported that a fermentation lasting longer than 6 days was not needed because at day 6 the residual NDF approached the asymptotic amount and recoveries of INDF for days 6, 7, and 8 did not differ.

In spite of the widespread use of indigestible fiber as

an internal marker, considerable variation in marker recovery has been noted (Cochran et al., 1987), with variation being trial and even diet dependent (Galyean et al., 1986). For example, INDF appeared to give a useful estimate of the digestibility of coastal bermudagrass and perennial ryegrass pastures when grazed by esophageally fistulated steers (Pond and Ellis, 1979). In contrast, variation among diets was demonstrated by Cochran et al. (1986b) in a trial utilizing four different diets. They demonstrated that steers consuming cubed alfalfa hay or mature tall wheatgrass had digestion coefficients for IADF and INDF ratio that were similar to the in vivo coefficients. However, estimates of tall fescue and prairie hay in the same trial significantly differed from in vivo values. Lippke et al. (1986) reported recoveries of INDF ranging from 83 to 111% for subtropical forages, 130% for immature ryegrass forage and 96% for sorghum forages. Additionally, other studies have shown that recoveries may be influenced by the level of grain supplement in the diet and suggests problems exist between laboratory techniques (Patterson and Kerly, 1987).

CHAPTER I

EVALUATION OF RUMINAL EVACUATION VERSUS MARKER DILUTION PROCEDURES FOR ESTIMATING PARTICLE PASSAGE RATES

Summary

Three experiments were conducted to evaluate ruminal evacuation versus marker dilution procedures for estimating particle passage rates. In Exp.1, eight ruminally fistulated steers (wt=527 kg) were fed alfalfa cubes at 2% of body weight (BW) and were assigned to two frequencies of feeding (FF): 1) once daily and 2) eight times daily. Ruminal dilution of Yb-labeled fecal particles, Dy-labeled alfalfa cubes and an aqueous solution of Er was monitored in each animal over a 48-h period. In Exp.2, eight ruminally fistulated heifers (wt=239 kg) were fed alfalfa cubes at 1.5% of BW and were assigned to two FF: 1) once daily and 2) twelve times daily. Ruminal and fecal curves of Yb-labeled alfalfa cubes were monitored in each animal for 48 and 96 h, respectively. In Exp.3, 16 ruminally fistulated steers (wt=409 kg) were fed dormant, bluestem-range grasses ad libitum and were assigned to four supplementation treatments. Ruminal and fecal curves of Yb-labeled range grass were monitored for 48 and 96 h, respectively. Indigestible acid detergent fiber (IADF) and alkaline peroxide lignin (APL) were used as reference

substances for calculating passage rates from ruminal evacuation measured at: Exp.1 = 12h postfeeding, Exp.2 = 0,3, and 12h postfeeding and Exp.3 = 0 and 4 h postfeeding. A technique x treatment interaction was evident (P=.01) for Exp.1 and 2 but not for Exp.3 (P=.20). For Exp.1 and 2, mean passage rates determined by evacuation were not influenced (P>.10) by frequency of feeding; however, ruminal marker curves yielded faster passage rates (P<.03) when animals were fed more frequently. For Exp.3, passage rates estimated via markers were similar to rates from evacuation when APL was used as the internal marker. Under most conditions, APL and IADF yielded similar passage rates via evacuation. Dry matter fill estimates from ruminal marker curves were significantly higher than those measured by evacuation. In conclusion, ruminal evacuation appears to be a viable approach for estimating particle passage, however, timing and number of evacuations should be considered.
(Key Words: Rate of Passage, Rumen Evacuation, Feeding Frequency, Markers.)

Introduction

Thorough depiction of the nutritional status of forage-consuming ruminants depends on accurate estimates of intake, fill and digesta flow characteristics. Several methods have been used to determine particulate passage and ruminal fill (Warner, 1981). Presently, kinetic analysis of marker dilution and(or) excretion curves has become widespread. However,

marker-based procedures have been suggested to be characterized by many problems including choice of mathematical model, marker migration and analytical difficulties (Uden et al., 1980; Mader et al., 1982; Coleman et al ., 1984;). Direct estimation of passage via ruminal evacuation has been used by some researchers (Paloheimo and Makela, 1959; Waldo, 1965; Poppi et al., 1981ab) and appears to avoid some methodology problems associated with marker procedures while offering a less time consuming measurement of passage and fill (Reid, 1965; Teeter and Owens, 1984). Additionally, ruminal evacuation appears to be a procedure that would be more suitable for estimating passage and fill under extensive grazing conditions. Therefore, the purpose of this study was to compare passage rates derived by marker dilution procedures with those obtained directly from measuring intake and fill of an indigestible substance. Additionally, effect of feeding frequency, supplementation and evacuation time on particle passage and ruminal fill estimates were examined.

Experimental Procedure

Three experiments were conducted to evaluate the potential of ruminal evacuation as a procedure for estimating particle passage rates and ruminal fill. In Exp. 1, eight ruminally fistulated Angus x Hereford steers (avg. initial wt = 527 kg) were fed alfalfa cubes (chemical composition Table 1) at 2%

of body weight (BW) and were assigned to two frequencies of feeding (FF): 1) once daily (1x) and 2) eight times daily (8x). Particle markers were: 1) Yb bound to fecal particles from alfalfa cubes by immersion (feces-Yb), 2) Dy bound to alfalfa cubes by immersion (feed-Dy), 3) Er in aqueous solution (Er-sol). The immersion technique as described by Teeter et al. (1984). On day 1 of the collection period, 210 g of labeled forage (3.3 g Dy) and 210 g of labeled feces (4.4 g Yb) were placed in the rumen of each animal at 0700h. Materials were stratified by placing approximately equal portions of the labeled substance at successively higher levels intraruminally, beginning with the mid-ventral region and stopping at the mid-dorsal region of the rumen. Additionally, 1.83 g of Er that had been put into solution .5h before dosing was placed on top of the hay mat after the labeled feed and feces had been dosed. Serial samples were collected from the rumen before dosing (0h) and at 4, 8, 12, 16, 24, 32, and 48h post-dosing. In all three experiments, samples were collected in the same order that steers were dosed and within 5 min of designated sampling time. Ruminal digesta samples were composites of subsamples collected from the dorsal, ventral, midcranial and midcaudal regions of the rumen. Fill was measured via ruminal evacuation (RE) 12h post-feeding on the same day that the final marker collection was completed. Evacuated ruminal contents were mixed, subsampled in triplicate and immediately returned to the animals. Fill

was also estimated by extrapolating ruminal marker curves to time zero and dividing the antilog of the intercept into the dose (expressed in ug).

In experiment 2, eight ruminally fistulated Angus x Hereford heifers (avg. initial wt = 239 kg) were fed alfalfa cubes (chemical composition in Table 1) at 1.5% of BW and 1 lb of a ground corn supplement per day. Heifers were assigned to two frequencies of feeding: 1) once daily (1x) and 2) twelve times daily (12x). The particulate marker, Yb-labeled alfalfa cubes, was prepared by the immersion procedure of Teeter et al. (1979) with the exception that four layers of cheesecloth were used to cover a 50 gallon tub, a hose was inserted into the bottom of the tub and water was continuously run for 24 h to remove loosely bound marker. On day 1 of the collection period, 250 g of the labeled forage (3.1 g Yb) was stratified in the rumen of each animal at 0700 h. Serial samples were collected from the rumen before dosing (0h) and at 4, 8, 12, 16, 24, 32 and 48h post-dosing. Fecal grab samples were also collected before dosing (0h) and at 4, 8, 12, 16, 20, 24, 28, 32, 40, 48, 60, 72, 84, and 96h post-dosing. Fecal samples were collected by inducing defecation by rubbing the dorsal rectal wall and saving the final 150 to 300g of feces excreted (Hartnell and Satter, 1979). Fill was measured via RE at 0, 3, and 12h post-feeding. Ruminal evacuations were begun on the same day that the final marker collection was completed, and were accomplished over three

successive days. On day 1, the 0h evacuation was accomplished at the same time as the 96h fecal collection (immediately before feeding). The 3 and 12 h evacuations were accomplished on d 2 and 3, respectively.

For experiment 3, sixteen ruminally fistulated Angus x Hereford steers (avg. initial wt = 409 kg) that were part of an ongoing trial (DelCurto et al., 1988) were used. Briefly, steers involved in this trial were blocked by weight and randomly assigned to treatments which were arranged in a 2x2 factorial structure consisting of daily supplementation with 2 levels of supplemental crude protein (CP) (.66g/kg body weight (BW) and 1.32 g/kg BW) and two levels of metabolizable energy (9.2 Kcal/ kg BW and 18.4 Kcal/kg BW). Treatments were: 1) low protein, low energy (LPLE) = 22% CP fed at .3% BW, 2) low protein, high energy (LPHE) = 11% CP fed at .6% BW, 3) high protein, low energy (HPLE) = 44% CP fed at .3% BW and 4) high protein, high energy (HPHE) = 22% CP fed at .6% BW (chemical composition in Table 2). Supplement was offered once daily at 0800 followed by a basal diet of dormant bluestem range-forage (chemical composition in Table 2) fed at 130% of the previous 5-d average intake. All range forage was from the same source and chopped to an average length of 3.5 cm. Feed remaining in feeders was removed and weighed immediately before the next feeding. Animals were individually housed in 1.53 m by 7.62 m with free access to water and a salt and trace-mineral block.

Ytterbium bound to the dormant bluestem range-forage by the same immersion technique as described for Exp. 2 was used as the particulate marker. On day 1 of the collection period, 250 g of the labeled forage (1.1 g Yb) was stratified in the rumen at 0700 h. Serial samples were collected from the rumen before dosing (0h) and at 4, 8, 12, 16, 24, 32, and 48h post-dosing. Fecal grab samples were also collected before dosing (0h) and at 4, 8, 12, 16, 20, 24, 28, 32, 39, 48, 60, 72, 84, and 96h post-dosing. Fill was measured via evacuation at 0 and 4h post-feeding. Ruminal evacuations were begun on the same day that the final marker collection was completed.

Dry matter intake was recorded daily during each experiment and subsamples of all feeds and orts were collected. Feed, orts, fecal and ruminal digesta samples were dried at 50 C in a forced air oven then ground with a Cyclotec Mill[1] to pass a 1 mm-screen. Duplicate samples were analyzed for dry matter (DM), ash and Kjeldahl N by standard procedures (AOAC, 1980). Samples in Exp. 3 were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to the procedures of Goering and Van Soest (1970).

Rare earth was extracted from samples with .05M EDTA by the procedure of Hart and Polan (1984). Rare earth concentrations in the extracts were determined by atomic absorption spectrophotometry (nitrous oxide/acetylene flame).

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[1]Tecator, Inc: P.O. Box 405, Herndon, Va 22070.

Common matrix standards were used in preparing standard curves. Passage rates from ruminal Yb, Dy, and(or) Er concentration curves were derived by semilogarithmic regression. Passage rates from fecal excretion curves were derived from the two compartment model of Grovum and Williams (1973). Rate of passage from RE was estimated by dividing intake of the indigestible reference substance by reticuloruminal mass of the same substance. Indigestible acid detergent fiber (IADF) and alkaline peroxide lignin (APL) were used as the indigestible reference substances. For Exp.2 and 3, passage was estimated for each time of ruminal evacuation and these individual rates were also averaged to represent a mean passage value from RE for both APL and IADF.

Isolation of indigestible ADF in feed, orts, ruminal and fecal residues was accomplished by the technique of Waller et al. (1980) as modified by Cochran et al. (1986). However, 50 ml plastic tubes fitted with bunsen valves were used in place of screw top glass tubes. Ruminal liquor for inoculum was collected via ruminal cannula from a Holstein steer maintained on a diet comprised of 60% alfalfa hay (sun-cured, mid-bloom) and 40% concentrate (primarily corn grain). Inoculum was collected approximately 4 h after feeding. Triplicate samples from all trials including standard forages, were handled simultaneously within a single, large run, with an in vitro fermentation period of 144h. Weights of indigestible ADF were recorded as the difference between crucible plus residue

before and after ashing and were expressed as a percentage of the original sample DM. Alkaline peroxide lignin was determined according to the procedures of Cochran et al. (1988) where the alkaline hydrogen peroxide treatment was accomplished before the acid detergent extraction step.

Particulate passage rates and rumen dry matter fill were analyzed as a split plot analysis of variance. For Exp. 1, terms included in the model were feeding frequency (FF), animal nested within FF, procedure for estimating passage rate and FF x procedure. Animal nested within FF was used as the testing term for FF. For Exp.2, terms included in the model were FF, block, FF x block, procedure, and FF x procedure. FF x block was used as the testing term for FF. For Exp.3, terms included in the model were block, protein, energy, procedure, protein x procedure, energy x procedure, protein x energy x procedure, block x protein x energy. Block x protein x energy was used as the testing term for protein, energy and the protein x energy interaction. In all experiments, means were separated using linear contrasts and all analyses were performed using the General Linear Models procedure of SAS (SAS, 1982).

Results and Discussion

Experiment 1. Passage rate constants (Table 3) produced by different procedures varied depending on the frequency of feeding (P=.01). Rate constants from feces-Yb (P=.01) and feed-Dy (P=.02) were larger for 8x steers compared to 1x.

These results differ from those of Coleman et al., (1984) who reported a faster passage rate for twice daily versus continuously fed steers; however, these authors noted that a slight rise in intake for the steers fed twice daily may have influenced their observations. Ruiz and Mowat (1987) reported that particulate passage rates estimated in cattle fed high forage rations ad libitum were not affected by offering feed either once or four times daily. In the present study, rate constants from sol-Er and RE methods were not influenced ($P>.10$) by FF. The APL and IADF procedures had similar ($P=.62$) rate constants regardless of FF and their average rate (2.0 %/h) was slower ($P\leq.05$) than the average of the feces-Yb and feed-Dy passage rate estimates.

Rate of passage estimates are intended to portray the irreversible escape of undigestible particles via the reticulo-omasal orifice (Van Soest, 1982). Labeling of undigested feed materials may result in bias due to potential digestive influences on labeled feeds. Labeling undigested residues (feces from a steer fed alfalfa cubes) in Exp. 1. did not result in a significant difference in rate constants compared with dosing alfalfa cubes that had been labeled directly. This observation suggests that the immersion/rinsing procedure employed resulted in the marker binding similar fragments for both feed and feces. In contrast, dosing with an aqueous solution of rare earth onto the top of the hay mat in the rumen resulted in faster passage rates ($P<.02$). It is

possible that some of the marker may have left the rumen more rapidly than normal due to the close proximity of the dosing site and the reticulo-omasal orifice. Distributing the marker solution throughout the rumen might yield results more similar to dosing labeled material.

Procedures for estimating rumen dry matter content interacted (P=.08) with FF (Table 4). Fill estimates from feces-Yb (P=.01) and sol-Er were larger (P=.04) for the 1x treatment compared with the 8x treatment. The Er-sol fill estimates were larger (P=.01) than those derived by other marker procedures. Dy-feed (P=.20) and RE dry matter fill (P=.73) estimates were not influenced by feeding frequency. Fill estimated by Dy-feed was larger (P=.06) compared with Yb-feces when steers were fed 8x daily but did not differ (P=.17) when steers were fed 1x daily. The average of fill estimates from the Yb-fecal and Dy-feed procedures were higher ($P \leq .02$) than by RE regardless of feeding frequency. Possible explanations for these results include: 1) incomplete Yb recovery by the EDTA procedure (Doran et al., 1988); 2) inadequancy of the mathematical approach used to estimate fill;

Exp. 2. Influence of different procedures on passage rate estimates depended (P=.01) on the frequency of feeding (Table 5). In agreement with Exp.1, the marker procedures yielded slower passage rate constants for the 1x versus the 12x heifers. The increased rate of passage with increased FF may

be a result of an increased water consumption and subsequently increased rate of small particle removal from the rumen (Ulyatt et al., 1984; Bunting et al., 1987). Additionally, the curve for Yb concentration versus time for the 12x treatment (figure 1) more closely follows the expected classical exponential decline than that of the 1x treatment (figure 2). This deviation from exponential decline was noted in Exp.1 as well. In contrast, rate constants calculated from fecal excretion curves in Exp.2. were similar across FF (P=.40) suggesting that passage rate estimated from fecal curves were less sensitive to FF changes than passage rates estimated from ruminal curves.

It has been suggested that more frequent feeding removes diurnal variation and under restricted feeding conditions results in more stable ruminal conditions (Ruiz and Mowat, 1987). Therefore, time of evacuation should not affect passage rate estimates in frequently fed ruminants. In Exp. 2, passage rate estimates within the 1x treatment derived via RE at 0, 3, and 12 h postfeeding differed significantly. Within the 12x treatment, passage rates from RE measured at the same times showed a greater degree of similarity, but continued to display some diurnal variation. However, when the passage rate constants were averaged across evacuation time, no FF effect was noted for RE. Mean passage rates from RE were similar (P>.60) for APL compared with IADF. Within the 1x treatment, ruminal and fecal Yb curves tended to yield smaller passage

rates than those derived from RE using APL (P=.11) and IADF (P=.22 and .23). In contrast, under the 12x feeding frequency marker dilution and RE procedures yielded similar (P>.50) passage rate estimates.

Influence of the procedure used on resulting estimates of rumen dry matter fill also depended (P=.01) on the frequency of feeding (Table 6). Within the 1x treatment, DM fill at 0h was lower (P=.02) than DM fill at 3h postfeeding. However, within the 12x treatment, ruminal evacuations at 0, 3, and 12 h yielded similar (P>.15) rumen dry matter fill values. Observations of the eating behavior of the two groups of heifers suggests that the 1x heifers ate the majority of their daily feed shortly after feeding, which probably accounts for most of the observed differences in DM fill between times. Fill estimates from ruminal marker curves were significantly higher than measured fill values at all evacuation times.

Exp. 3. Influence of procedure on passage rate estimates did not depend (P=.20) on the supplementation treatments used (Table 7). Additionally, time of evacuation in Exp.3 did not have a marked effect on particulate passage rate estimates which is in contrast to results found in Exp. 2.

Hartnell and Satter (1979) suggested that constructing marker curves via fecal grab samples may be more desirable due to mixing and sampling problems associated with ruminal sampling; however, passage rates in Exp.3 estimated from

ruminal and fecal marker curves were similar (P=.64). Staples et al. (1984) also observed similar rate constants for fecal and ruminal curves. Passage rates estimated from marker curves did not differ (P>.60) compared with the mean rate from RE when APL was used as the marker. Mean passage rate constants from RE were .2 to .4% /h higher (P<.08) when IADF was used as the internal marker. Considerable variation in IADF recovery has been noted (Cochran et al., 1987) with variation in recovery being both trial and diet dependent (Galyean et al., 1986). Cochran et al., (1986b) found that steers fed cubed alfalfa hay, as in Exp. 1 and 2 displayed virtually quantitative recovery of IADF, however recovery on a prairie hay diet, as in Exp. 3, was less than quantitative. Marker recovery may also be influenced by level of grain supplementation (Patterson and Kerly, 1987). Initial evaluation of alkaline peroxide lignin (where peroxide treatment was accomplished before acid detergent extraction) indicates this internal marker exhibits promise as an indigestible reference substance for bluestem range diets such as that used in this experiment (Cochran et al., 1988). Differences in marker recovery may explain some of the differences in rate constants observed in this experiment.

Influence of procedure on estimates of rumen DM fill depended (P=.03) on the level of supplemental protein offered (Table 8). Ruminal DM fill tended to be greater when supplemented with high levels of protein when fill was

estimated by ruminal marker curves (P=.12) or was measured by RE at 4h postfeeding (P=.12). This is in agreement with DelCurto et al (1987) who noted increased DM fill for steers supplemented with moderate and high levels of protein. This increase in fill corresponded with an increased DM intake, which would account for the lack of difference in passage rate constants with supplementation treatment. When fill was measured by RE at 0h postfeeding no difference was evident in fill levels (P=.25) for high versus low protein supplementation treatments. In agreement with Exp. 1 and 2, ruminal DM fill was larger (P=.01) when estimated from ruminal marker curves compared with RE.

In conclusion, although there were minor differences evident in the passage rate constants estimated from RE vs rare earth marker procedures, RE appears to be a viable alternative to rare earth markers if careful consideration is given to time of ruminal evacuation. Evacuation time will exert the most dramatic influence on passage rate constants from RE when cattle are fed infrequently. If feeding frequencies and feeding behavior are relatively similar among treatments, choice of evacuation time may be less critical. Only minor differences in rate constants were evident when IADF and APL were used as the internal markers for estimating passage via rumen evacuation. In all cases, dry matter fill was significantly over-estimated by the marker curves, indicating either that they are unsuitable under these

conditions, a different extraction method or a different mathematical approach may be needed for estimating fill when using a simple exponential model.

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TABLE 1. CHEMICAL COMPOSITION OF ALFALFA CUBES AND SUPPLEMENTS USED IN EXPERIMENTS 1 AND 2

	Component %[a]		
Item	CP[b]	IADF[b]	APL[b]
Alfalfa cubes (Exp.1)	20.9	24.4	6.2
Alfalfa cubes (Exp.2)	22.3	29.9	7.6
Grain supplement (Exp.2)	11.4	3.7	.8

[a]Organic matter basis

[b]CP = crude protein, IADF = indigestible acid detergent fiber, APL = alkaline peroxide lignin.

TABLE 2. CHEMICAL COMPOSITION OF DORMANT HAY AND SUPPLEMENTS USED IN EXPERIMENT 3

	Component % (organic matter basis)				
Item	CP[a]	NDF[a]	ADF[a]	IADF[a]	APL[a]
Dormant prairie hay	3.1	86.7	59.5	43.5	5.2
Treatment supplement:					
1. LPLE[b]	22.8	13.4	7.3	3.4	.30
2. LPHE[b]	11.8	10.0	5.3	2.7	.40
3. HPLE[b]	44.8	19.2	19.2	5.1	.31
4. HPHE[b]	22.8	13.4	7.3	3.4	.30

[a]CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber IADF = indigestible acid detergent fiber, APL = alkaline peroxide lignin

[b]LPLE = low protein, low energy; LPHE = low protein, high energy; HPLE = high protein, low energy; HPHE = high protein, high energy.

TABLE 3. PASSAGE RATE CONSTANTS ESTIMATED BY DIFFERENT TECHNIQUES IN STEERS FED AT TWO FREQUENCIES (EXP. 1)

	..... Technique[a]					
Item	Yb-labeled feces	Dy-labeled feed	Er solution	APL[b]	IADF[b]	SE[c]
			.....(h^{-1}).....			
Frequency of feeding[d]						
1X	2.2	2.6	3.3	1.9	2.0	.2
8X	3.4	3.5	4.2	2.0	2.2	.2
SE[c]	.2	.2	.4	.2	.1	
Probability level (FF)	P=.01	P=.02	P=.16	P=.49	P=.35	

[a]Technique x FF interaction (P=.01)

[b]APL= alkaline peroxide lignin, IADF= indigestible acid detergent fiber. Passage rate constants using internal markers were estimated by the ratio of marker consumed per unit time to ruminal mass of marker.

[c]Standard error (n=4)

[d]Linear contrasts. Probability of significant effect:

	1X	8X
APL VS IADF	.62	.62
Dy vs Yb	.17	.94
(Dy + Yb/2) vs Er	.01	.02
(Dy +Yb) vs (APL + IADF)	.05	.01

TABLE 4. RUMEN DRY MATTER CONTENTS ESTIMATED BY DIFFERENT TECHNIQUES IN STEERS FED AT TWO FREQUENCIES (EXP. 1)

	Technique[a]				
Item	Yb-labeled feces[b]	Dy-labeled feed[b]	Er Solution[b]	RE[c]	SE[d]
	Mass (kg)				
Frequency of feeding[e]					
1X	12.9	14.6	18.8	9.4	.8
8X	10.7	12.9	15.2	9.0	.8
SE[d]	.4	.8	1.0	.8	
Probability level (FF)	P=.01	P=.20	P=.04	P=.73	

[a]Technique x FF interaction (P=.08)

[b]Mass (g)= (dose (g) x 1,000,000) / $e^{intercept}$

[c]RE= Dry matter fill estimated by rumen evacuation.

[d]Standard error (n=4)

[e]Linear contrasts. Probability of significant effect:

	1X	8X
Yb vs Dy	.17	.06
(Yb + Dy/2) vs Er	.01	.01
(Yb + Dy/2) vs RE	.01	.02

TABLE 5. PASSAGE RATE CONSTANTS ESTIMATED BY DIFFERENT TECHNIQUES IN HEIFERS FED AT TWO FREQUENCIES (EXP. 2)

Procedure[b]	Frequency of feeding (FF)[a] 1 X	12 X	SE[c]	Probability level
	.....(h^{-1}).....			
IADF-0h[d]	7.5	5.4	.6	.10
IADF-3h	3.4	4.4	.2	.06
IADF-12h	4.3	5.7	.6	.21
IADF-Mean	5.1	5.2	.4	.86
APL-0h[d]	7.8	4.9	.5	.02
APL-3h	3.5	4.4	.3	.11
APL-12h	4.4	6.1	.8	.23
APL-Mean	5.2	5.3	.4	.90
Ruminal-Yb	4.6	5.4	.2	.03
Fecal-Yb	4.6	4.9	.3	.40
SE[c]	.4	.4		

[a]Technique x FF interaction (P=.01)

[b]Linear contrasts. Probability of significant effect:

	1x	12x
APL-mean vs IADF-mean	.68	.83
Fecal-Yb vs Ruminal-Yb	.97	.42
APL-mean vs Ruminal-Yb	.11	.84
APL-mean vs Fecal-Yb	.11	.54
IADF-mean vs Ruminal-Yb	.22	.68
IADF-mean vs Fecal-Yb	.23	.69
APL-0h vs APL-3h	.01	.45
APL-0h vs APL-12h	.01	.05
APL-3h vs APL-12h	.03	.01
IADF-0h vs IADF-3h	.01	.11
IADF-0h vs IADF-12h	.01	.66
IADF-3h vs IADF-12h	.05	.04

[c]Standard error (n=4)

[d]APL= alkaline peroxide lignin, IADF= indigestible acid detergent fiber. Passage rate constants using internal markers were estimated by the ratio of marker consumed per unit time to ruminal mass of marker.

TABLE 6. RUMEN DRY MATTER CONTENTS ESTIMATED BY DIFFERENT TECHNIQUES IN HEIFERS FED AT TWO FREQUENCIES (Exp. 2)

Procedure	Frequency of feeding[a] 1X	12X	SE[b]	Probability level
	.....Mass (kg).....			
DM Fill-0h[c]	1.9	3.0	.2	.03
DM Fill-3h	5.1	3.2	.2	.01
DM Fill-12h	3.8	2.8	.2	.04
Mean DM Fill	3.6	3.0	.2	.06
Yb ruminal fill[d]	16.0	10.6	1.8	.13
SE[b]	.9	.2		
	....Probability level....			
Mean DM vs Yb-Ruminal	.01	.01		
DM-0h vs Yb-Ruminal	.01	.01		
DM-3h vs Yb-Ruminal	.01	.01		
DM-12h vs Yb-Ruminal	.01	.01		
DM-12h vs DM-0h	.15	.57		
DM-0h vs DM-3h	.02	.36		
DM-12h vs DM-3h	.27	.15		

[a]Technique x FF interaction (P=.01)

[b]Standard error (n=4)

[c]Dry matter fill estimated by rumen evacuation at 0, 3, and 12h postfeeding

[d]Mass (g) = (Dose (g) x 1,000,000) / $e^{intercept}$

TABLE 7. PASSAGE RATE CONSTANTS ESTIMATED BY DIFFERENT TECHNIQUES IN STEERS RECEIVING DORMANT BLUESTEM RANGE-GRASS AND SUPPLEMENTED WITH DIFFERENT LEVELS OF PROTEIN AND ENERGY (EXP. 3)

Procedure[a]	Passage rate (h^{-1})	SE[b]
APL-0h[c]	1.72	.11
APL-4h	1.78	.12
APL-Mean	1.72	.11
IADF-0h[c]	2.17	.11
IADF-4h	2.01	.11
IADF-Mean	2.11	.11
Yb-Fecal	1.80	.11
Yb-Ruminal	1.71	.11

[a]Linear contrasts. Probability of significant effect:

APL-Mean vs IADF-Mean	.03
Yb-Rumen vs Yb-Fecal	.64
APL-Mean vs Yb-Rumen	.97
APL-Mean vs Yb-Fecal	.66
IADF-Mean vs Yb-Rumen	.03
IADF-Mean vs Yb-Fecal	.08

[b]Standard error (n=16)

[c]APL= alkaline peroxide lignin, IADF=indigestible acid detergent fiber. Passage rate constants using internal markers were estimated by the ratio of marker consumed per unit time to ruminal mass of marker.

TABLE 8. RUMEN DRY MATTER CONTENTS ESTIMATED BY DIFFERENT TECHNIQUES IN STEERS RECEIVING DORMANT RANGE-GRASS AND SUPPLEMENTED WITH DIFFERENT LEVELS OF PROTEIN AND ENERGY (Exp. 3)

	Protein level[a]			
Procedure	HP	LP	SE[b]	Probability level
DM Fill-0h[c]	9.7	8.8	.5	.25
DM Fill-4h	12.1	10.7	.6	.12
Yb Ruminal fill[d]	28.8	19.7	3.7	.12
	...Probability level...			
DM Fill-0h vs DM Fill-4h	.35	.21		
DM Fill-0h vs Yb fill	.01	.01		
DM Fill-4h vs Yb fill	.01	.01		
DM Fill 0+4/2 vs Yb fill	.01	.01		

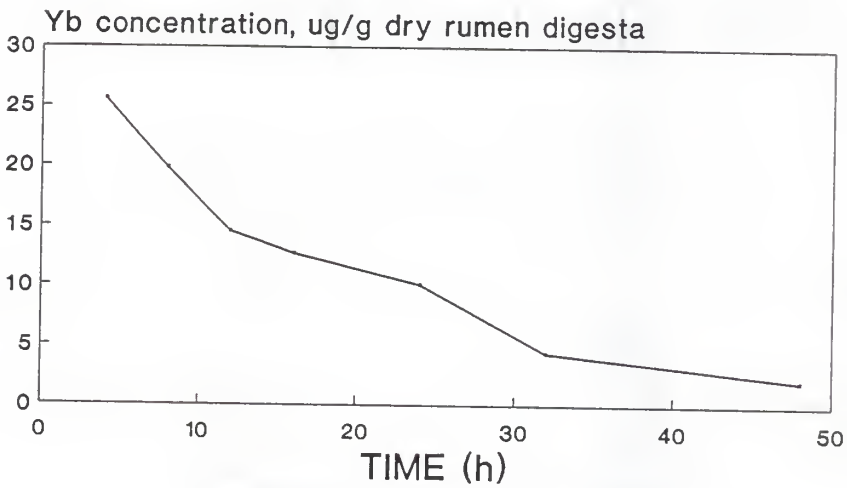
[a]Protein x procedure interaction (P=.03)

[b]Standard error (n=8)

[c]Dry matter fill estimted from rumen evacuation at 0 and 4h postfeeding

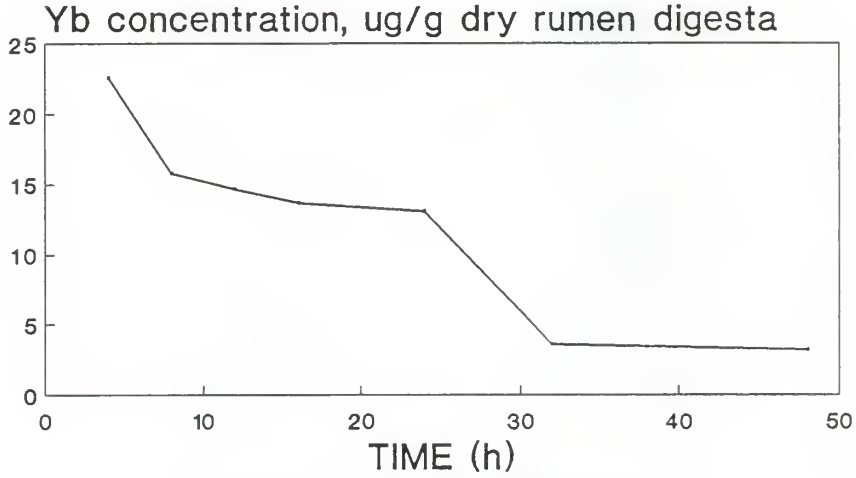
[d]Mass (g) = (dose (g) x 1,000,000) / $e^{intercept}$

Figure 1. Ruminal marker concentration curve for heifers fed alfalfa cubes twelve times daily (Exp.2)a



a Each point represents the average of four observations.

Figure 2. Ruminal marker concentration curve in heifers fed alfalfa once daily (Exp.2)a



a Each point represents the average of four observations.

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APPENDIX A

APPENDIX A

RUMINAL RARE EARTH PROFILES FOR STEERS WITHIN DIFFERENT TREATMENT GROUPS IN THE PARTICLE PASSAGE EXPERIMENT CONDUCTED AT MILES CITY, MONTANA (EXP. 1).

TABLE A1. RUMINAL Yb CONCENTRATION FOR RUMINALLY SAMPLED STEERS FED 1X DAILY (EXPERIMENT 1).

	Steer Identification			
Time	057	774	342	815
	.....(ug/g dry rumen digesta).....			
4	260.2	292.3	255.1	302.2
8	243.5	236.4	240.3	269.4
12	256.0	260.2	291.4	284.7
16	293.3	277.7	299.8	292.9
24	286.9	308.3	298.3	323.2
32	112.2	148.9	103.0	102.9
48	122.5	140.3	121.5	100.5

TABLE A2. RUMINAL Yb CONCENTRATION FOR RUMINALLY SAMPLED STEERS FED 8X DAILY. (EXPERIMENT 1).

	Steer Identification			
Time	240	028	620	382
	.....(ug/g dry rumen digesta).....			
4	316.7	364.7	325.5	356.0
8	299.7	307.0	284.5	321.5
12	273.7	303.5	286.8	311.4
16	226.4	236.6	238.3	232.6
24	191.8	221.2	198.2	184.8
32	142.1	137.3	126.7	131.1
48	75.6	80.1	80.4	71.0

TABLE A3. RUMINAL Dy CONCENTRATION FOR RUMINALLY SAMPLED STEERS FED 1X DAILY (EXPERIMENT 1).

	Steer Identification			
Time	057	774	342	815
	.....(ug/g dry rumen digesta).....			
4	137.8	169.2	158.2	196.4
8	142.1	154.1	153.4	178.0
12	148.5	185.1	206.0	183.0
16	161.9	186.8	218.5	222.2
24	171.1	185.0	191.9	251.4
32	73.4	77.1	62.1	56.6
48	56.1	75.1	60.8	55.3

TABLE A4. RUMINAL Dy CONCENTRATION FOR RUMINALLY SAMPLED STEERS FED 8X DAILY (EXPERIMENT 1).

	Steer Identification			
Time	240	028	620	382
	.....(ug/g dry rumen digesta.....			
4	204.4	238.1	193.3	228.9
8	193.0	166.0	188.0	200.9
12	162.2	182.1	176.1	199.1
16	156.0	138.9	147.1	146.6
24	131.2	133.7	127.0	114.9
32	81.2	81.4	71.0	70.6
48	45.4	50.1	50.3	50.7

TABLE A5. RUMINAL Er CONCENTRATION FOR RUMINALLY SAMPLED STEERS FED 1X DAILY (EXPERIMENT 1).

	Steer Identification			
Time	057	774	342	815
	.....(ug/g dry rumen digesta).....			
4	61.2	71.8	76.5	60.5
8	60.9	51.4	51.2	61.0
12	81.9	115.1	90.5	81.3
16	70.9	70.7	76.3	60.6
24	65.5	66.8	60.7	77.0
32	20.4	25.7	20.7	20.6
48	20.4	30.0	15.2	15.1

TABLE A6. RUMINAL Er CONCENTRATION FOR RUMINALLY SAMPLED STEERS FED 8X DAILY. (EXPERIMENT 1).

	Steer Identification			
Time	240	028	620	382
	.....(ug/g dry rumen digesta).....			
4	51.1	101.3	127.1	91.6
8	96.5	85.6	116.8	115.5
12	76.0	75.8	75.5	66.4
16	65.4	61.7	71.0	60.7
24	45.4	41.2	40.7	30.0
32	35.6	40.7	35.5	25.2
48	15.1	20.0	20.1	10.1

APPENDIX B

APPENDIX B

FECAL AND RUMINAL Yb PROFILES FOR HEIFERS FED ALFALFA CUBES ONCE OR TWELVE TIMES DAILY IN PARTICLE PASSAGE EXPERIMENT 2.

TABLE B1. FECAL Yb CONCENTRATIONS FOR RECTALLY SAMPLED HEIFERS FED 1X DAILY (EXPERIMENT 2).

	Heifer Identification			
Time	602	611	625	631
	.....(ug/g dry feces).....			
4	.3	.4	.4	.3
8	.6	.9	.4	1.9
12	1.4	2.4	1.5	2.4
16	9.4	14.5	9.0	11.5
20	18.2	26.5	18.8	22.0
24	24.9	28.6	24.8	25.6
28	23.4	24.6	23.4	25.5
32	27.6	25.9	27.5	24.3
40	19.3	19.6	20.8	20.4
48	15.0	16.6	17.1	17.2
60	11.5	13.5	17.4	18.8
72	2.5	5.5	2.7	4.1
84	1.8	2.1	2.0	2.8
96	1.3	1.4	2.0	1.5

TABLE B2. FECAL Yb CONCENTRATIONS FOR RECTALLY SAMPLED HEIFERS FED 12X DAILY (EXPERIMENT 2).

	Heifer Identification			
Time	606	607	609	619
	.....(ug/g dry feces).....			
4	.3	.3	.4	.3
8	.5	.6	.4	.3
12	2.3	2.3	1.3	1.0
16	8.8	7.7	8.4	6.4
20	22.5	21.8	26.4	15.3
24	30.8	29.1	27.9	26.2
28	26.4	28.9	24.6	27.4
32	31.3	27.2	27.2	31.0
40	22.9	20.3	21.4	25.5
48	15.6	14.9	18.2	16.5
60	11.9	10.9	13.1	14.8
72	2.9	3.3	3.1	2.5
84	2.0	2.0	2.0	1.8
96	1.7	1.6	1.6	1.4

TABLE B3. RUMINAL Yb CONCENTRATION FOR RUMINALLY SAMPLED HEIFERS FED 1X DAILY (EXPERIMENT 2).

	Steer Identification			
Time	602	611	625	631
	.....(ug/g dry rumen digesta).....			
4	17.7	26.2	23.4	23.2
8	15.6	17.8	15.5	14.4
12	15.5	15.1	14.0	14.3
16	12.8	14.3	13.8	14.0
24	12.6	11.4	14.8	13.5
32	3.3	3.5	3.0	4.5
48	3.1	3.0	1.6	5.0

TABLE B4. RUMINAL Yb CONCENTRATION FOR RUMINALLY SAMPLED HEIFERS FED 12X DAILY (EXPERIMENT 2).

	Steer Identification			
Time	606	607	609	619
	.....(ug/g dry rumen digesta).....			
4	31.3	21.4	25.7	23.9
8	18.8	15.1	24.0	21.3
12	15.5	12.5	14.4	15.5
16	12.8	10.9	11.3	15.8
24	12.3	9.4	8.8	9.8
32	6.7	3.5	3.7	3.5
48	2.7	2.2	2.6	.9

APPENDIX

C

APPENDIX C

FECAL AND RUMINAL Yb CONCENTRATIONS FOR STEERS WITHIN DIFFERENT TREATMENT GROUPS IN PARTICLE PASSAGE EXPERIMENT 3 (PROTEIN X ENERGY TRIAL).

TABLE C1. FECAL Yb CONCENTRATIONS FOR RECTALLY SAMPLED STEERS RECEIVING THE LOW ENERGY, LOW PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP.3).

	Steer Identification			
Time	71	86	102	118
	.....(ug/g dry feces).....			
4	0	0	.4	0
8	0	0	.6	0
12	.5	.4	.8	.5
16	.9	.7	.9	.9
20	1.0	1.4	.9	.9
24	1.2	.9	.8	1.0
28	2.1	1.5	1.3	1.7
32	2.7	2.0	1.4	2.3
39	3.1	3.6	2.5	3.2
48	4.1	3.5	3.4	4.5
60	3.6	2.8	2.3	3.2
72	2.9	2.9	2.6	2.9
84	2.7	2.2	2.4	2.8
96	1.8	1.7	1.5	2.1

TABLE C2. FECAL Yb CONCENTRATIONS FOR RECTALLY SAMPLED STEERS RECEIVING THE HIGH ENERGY, LOW PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP.3).

	Steer Identification			
Time	53	63	112	128
	.....(ug/g dry feces).....			
4	0	.4	.5	0
8	0	.6	.6	0
12	.7	.8	.7	0
16	1.0	.9	.7	.6
20	1.1	.9	1.0	1.2
24	1.1	1.1	1.2	1.5
28	1.6	1.5	1.3	1.9
32	1.9	1.7	1.4	2.7
39	3.0	2.1	2.6	3.9
48	3.6	2.9	2.8	2.8
60	2.6	2.8	2.5	5.0
72	2.4	2.6	2.5	5.3
84	2.2	2.5	2.4	5.1
96	1.7	2.0	1.4	2.3

TABLE C3. FECAL Yb CONCENTRATIONS FOR RECTALLY SAMPLED STEERS RECEIVING THE HIGH PROTEIN AND LOW ENERGY SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP. 3)

	Steer Identification			
Time	64	65	127	529
	.....(ug/g dry feces).....			
4	0	0	0	-
8	0	.4	0	-
12	.6	.4	.7	.6
16	.8	.9	.9	.8
20	1.0	1.1	.9	1.0
24	1.0	1.3	.6	1.1
28	1.5	1.1	1.0	1.9
32	1.8	1.5	1.5	2.2
39	1.7	2.5	2.7	3.5
48	2.5	2.6	2.9	3.4
60	2.7	2.9	3.5	3.1
72	2.0	2.2	2.6	1.8
84	1.4	1.6	2.4	1.5
96	1.0	1.5	1.4	1.2

TABLE C4. FECAL Yb CONCENTRATIONS FOR RECTALLY SAMPLED STEERS RECEIVING THE HIGH ENERGY AND HIGH PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP.3).

	Steer Identification			
Time	52	105	131	X83
	.....(ug/g dry feces).....			
4	0	0	0	00
8	.4	0	0	.5
12	.4	0	0	.6
16	.6	.6	.6	.8
20	.9	1.0	1.3	.9
24	1.0	.9	1.0	1.1
28	1.0	1.4	1.4	1.8
32	1.2	1.7	1.3	2.0
39	2.0	3.1	1.9	3.4
48	2.7	3.5	2.4	3.5
60	2.5	3.2	2.1	2.8
72	2.2	2.8	2.3	2.4
84	1.9	1.7	1.8	2.1
96	1.7	1.2	1.3	1.1

TABLE C5. RUMINAL Yb CONCENTRATION FOR RUMINALLY SAMPLED STEERS RECEIVING THE LOW ENERGY AND LOW PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP 3)

	Steer Identification			
Time	71	86	102	118
	.....(ug/g dry rumen digesta).....			
4	6.9	6.0	5.4	4.5
8	5.2	4.2	4.9	4.9
12	6.9	3.5	5.2	4.4
16	5.3	2.7	4.1	4.8
24	6.2	2.5	4.8	4.3
32	3.1	2.0	2.3	3.4
48	3.1	2.1	2.6	2.2

TABLE C6. RUMINAL Yb CONCENTRATION FOR RUMINALLY SAMPLED STEERS RECEIVING THE HIGH ENERGY, LOW PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP 3)

	Steer Identification			
Time	53	63	112	128
	(ug/g dry rumen digesta)			
4	8.1	3.6	5.2	5.4
8	6.8	4.5	4.2	5.3
12	6.0	3.1	3.0	6.2
16	4.8	4.5	3.9	11.0
24	4.5	3.6	3.1	10.2
32	1.9	1.2	2.8	3.9
48	2.2	2.8	2.3	5.3

TABLE C7. RUMINAL Yb CONCENTRATION FOR RUMINALLY SAMPLED STEERS RECEIVING LOW ENERGY, HIGH PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP 3).

	Steer Identification			
Time	64	65	127	529
	(ug/g dry rumen digesta)			
4	4.7	3.5	3.9	5.8
8	3.4	3.2	4.0	4.7
12	3.4	3.3	4.5	4.2
16	4.0	3.9	4.4	3.7
24	3.4	3.6	4.1	4.7
32	2.4	2.7	2.2	2.9
48	2.1	1.8	2.1	2.8

TABLE C8. RUMINAL Yb CONCENTRATION FOR RUMINALLY SAMPLED STEERS RRECEIVING THE HIGH ENERGY, AND HIGH PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP 3).

	Steer Identification			
Time	52	105	131	X83
	.....(ug/g dry rumen digesta).....			
4	1.0	3.0	7.1	4.2
8	1.8	3.7	4.3	3.5
12	3.5	4.0	3.6	4.8
16	3.1	4.5	3.2	3.8
24	3.4	3.9	2.4	2.8
32	2.8	2.1	2.4	2.6
48	2.7	2.0	2.4	2.1

APPENDIX D

APPENDIX D

PASSAGE RATE CONSTANTS FROM RUMEN EVACUATION PROCEDURES FOR ANIMALS WITHIN DIFFERENT TREATMENT GROUPS FOR ALL THREE PARTICLE TURNOVER EXPERIMENTS

TABLE D1. PASSAGE RATE CONSTANTS FROM RUMEN EVACUATION PROCEDURES FOR STEERS FED 1X DAILY (EXPERIMENT 1).

	Steer Identification			
Procedure	057	774	342	815
APL	1.61	1.82	1.87	2.20
IADF	1.95	1.94	2.00	2.25

TABLE D2. PASSAGE RATE CONSTANTS FROM RUMEN EVACUATION PROCEDURES FOR STEERS FED 8X DAILY (EXPERIMENT 1).

	Steer Identification			
Procedure	240	028	620	382
APL	1.91	1.60	2.23	2.39
IADF	1.98	1.93	2.50	2.38

TABLE D3. PASSAGE RATE CONSTANTS FROM RUMEN EVACUATION PROCEDURES FOR HEIFERS FED 1X DAILY (EXPERIMENT 2).

	Steer Identification			
Procedure	602	611	625	631
APL-3h	4.07	3.75	3.40	2.78
APL-12h	5.24	4.40	5.00	2.99
APL-23h	7.76	8.25	9.74	5.44
APL-mean	5.69	5.47	6.05	3.74
IADF-3h	3.93	3.87	3.39	2.60
IADF-12h	4.67	4.39	4.83	3.27
IADF-23h	8.46	7.41	8.66	5.33
IADF-mean	5.69	5.22	5.63	3.73

TABLE D4. PASSAGE RATE CONSTANTS FROM RUMEN EVACUATION PROCEDURES FOR HEIFERS FED 12X DAILY (EXPERIMENT 2).

	Steer Identification			
Procedure	606	607	609	619
APL-3h	3.83	3.90	4.73	5.23
APL-12h	3.89	5.13	5.44	10.10
APL-23h	4.40	3.56	4.52	7.07
APL-mean	4.04	4.51	4.90	7.47
IADF-3h	4.03	4.19	4.46	5.03
IADF-12h	3.79	4.99	5.13	8.89
IADF-23h	4.42	4.20	4.96	8.15
IADF-mean	4.08	4.46	4.85	7.36

TABLE D5. PASSAGE RATE CONSTANTS FROM RUMEN EVACUATION PROCEDURES FOR STEERS RECEIVING THE LOW ENERGY, LOW PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP 3)

	Steer Identification			
Procedure	71	86	102	118
APL-3h	2.30	1.60	1.60	1.30
APL-23h	2.40	1.50	1.90	1.10
APL-mean	2.35	1.55	1.75	1.20
IADF-3h	2.17	1.94	2.19	1.96
IADF-23H	2.57	1.96	2.20	1.67
IADF-mean	2.37	1.95	2.20	1.82

TABLE D6. PASSAGE RATE CONSTANTS FROM RUMEN EVACUATION PROCEDURES FOR STEERS RECEIVING HIGH ENERGY AND LOW PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP 3)

	Steer Identification			
Procedure	53	63	112	128
APL-3h	1.50	1.40	1.60	2.20
APL-23h	1.20	2.40	1.40	1.00
APL-mean	1.35	1.90	1.50	1.60
IADF-3h	2.16	2.30	1.23	1.18
IADF-23h	2.20	1.91	1.10	1.85
IADF-mean	2.18	2.11	1.16	1.51

TABLE D7. PASSAGE RATE CONSTANTS FROM RUMEN EVACUATION PROCEDURES FOR STEERS RECEIVING THE LOW ENERGY, HIGH PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP 3)

	Steer Identification			
Procedure	64	65	127	529
APL-3h	1.80	1.60	1.90	1.40
APL-23h	2.00	1.40	1.90	2.50
APL-mean	1.90	1.50	1.90	1.95
IADF-3h	2.77	2.09	2.46	2.20
IADF-23h	2.53	1.91	2.22	2.12
IADF-mean	2.65	2.00	2.34	2.16

TABLE D8. PASSAGE RATE CONSTANTS FROM RUMEN EVACUATION PROCEDURES FOR STEERS RECEIVING THE HIGH ENERGY, HIGH PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP 3)

	Steer Identification			
Procedure	52	105	131	x83
APL-3h	1.90	2.20	1.70	1.60
APL-23h	2.00	2.00	1.20	2.00
APL-mean	1.95	2.10	1.45	1.80
IADF-3h	2.45	2.65	1.82	2.71
IADF-23h	2.06	2.67	1.40	2.16
IADF-mean	2.56	2.66	1.61	2.4

APPENDIX E

APPENDIX E

RUMIN DRY MATTER FILL FROM RUMIN EVACUATION FOR ANIMALS WITHIN DIFFERENT TREATMENT GROUPS IN THE PARTICLE PASSAGE STUDIES

TABLE E1. RUMEN DRY MATTER FILL DATA FOR STEERS FED 1X DAILY IN EXPERIMENT 1.

	Steer Identification			
Time	057	774	342	815
	.....kg.....			
3-h	11.05	9.66	9.46	7.41

TABLE E2. RUMEN DRY MATTER FILL DATA FOR STEERS FED 8X DAILY IN EXPERIMENT 1.

	Steer Identification			
Time	240	028	620	382
	.....kg.....			
3-h	10.22	....	9.08	7.72

TABLE E3. RUMEN DRY MATTER FILL DATA FOR HEIFERS FED 1X DAILY IN EXPERIMENT 2.

	Heifer Identification			
Time	602	611	625	631
		.....kg.....		
3-h	5.48	4.08	5.34	5.66
12-h	3.62	3.77	3.54	4.09
23-h	1.90	1.66	1.72	2.46
mean	3.67	3.17	3.53	4.07

TABLE E4. RUMEN DRY MATTER FILL DATA FOR HEIFERS FED 12X DAILY IN EXPERIMENT 2.

	Steer Identification			
Time	606	607	609	619
		.....kg.....		
3-h	3.10	3.68	3.04	3.12
12-h	3.64	2.96	2.69	1.78
23-h	3.30	3.64	2.68	2.17
mean	3.26	3.43	2.80	2.36

TABLE E5. RUMEN DRY MATTER FILL DATA FOR STEERS RECEIVING TH LOW ENERGY, LOW PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP 3)

	Steer Identification			
Time	71	86	102	118
		.....kg.....		
3-h	9.20	13.15	10.39	9.72
23-h	6.55	10.56	8.86	7.88

TABLE E6. RUMEN DRY MATTER FILL DATA FOR STEERS RECEIVING THE HIGH ENERGY, LOW PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXP 3)

	Steer Identification			
Time	53	63	112	128
		.....kg.....		
3-h	10.6	10.8	12.8	8.9
23-h	8.8	9.2	9.3	9.6

TABLE E7. RUMEN DRY MATTER FILL DATA FOR STEERS RECEIVING THE LOW ENERGY, HIGH PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXPERIMENT 3).

	Steer Identification			
Time	64	65	127	529
		.....kg.....		
3-h	11.8	13.2	11.8	11.5
23-h	8.9	12.4	9.6	9.7

TABLE E8. RUMEN DRY MATTER FILL DATA FOR STEERS RECEIVING THE HIGH ENERGY, HIGH PROTEIN SUPPLEMENT IN THE PROTEIN X ENERGY TRIAL (EXPERIMENT 3).

	Steer Identification			
Time	52	105	131	X83
		.....kg.....		
3-h	13.4	10.3	13.8	10.8
23-h	9.3	8.5	11.8	7.5

EVALUATION OF RUMINAL EVACUATION VERSUS MARKER DILUTION PROCEDURES FOR ESTIMATING PARTICULATE PASSAGE RATE

by

ALISON ANN BEHARKA

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Three experiments were conducted to evaluate ruminal evacuation versus marker dilution procedures for estimating particle passage rates. In Exp.1, eight ruminally fistulated steers (wt=527 kg) were fed alfalfa cubes at 2% of body weight (BW) and were assigned to two frequencies of feeding (FF): 1) once daily and 2) eight times daily. Ruminal dilution of Yb-labeled fecal particles, Dy-labeled alfalfa cubes and an aqueous solution of Er was monitored in each animal over a 48-h period. In Exp.2, eight ruminally fistulated heifers (wt=239 kg) were fed alfalfa cubes at 1.5% of BW and were assigned to two FF: 1) once daily and 2) twelve times daily. Ruminal and fecal curves of Yb-labeled alfalfa cubes were monitored in each animal for 48 and 96 h, respectively. In Exp.3, 16 ruminally fistulated steers (wt=409 kg) were fed dormant, bluestem-range grasses ad libitum and were assigned to four supplementation treatments. Ruminal and fecal curves of Yb-labeled range grass were monitored for 48 and 96 h, respectively. Indigestible acid detergent fiber (IADF) and alkaline peroxide lignin (APL) were used as reference substances for calculating passage rates from ruminal evacuation measured at: Exp.1 = 12h postfeeding, Exp.2 = 0,3, and 12h postfeeding and Exp.3 = 0 and 4 h postfeeding. A technique x treatment interaction was evident (P=.01) for Exp.1 and 2 but not for Exp.3 (P=.20). For Exp.1 and 2, mean passage rates determined by evacuation were not influenced (P>.10) by frequency of feeding; however, ruminal marker curves yielded faster passage rates (P<.03) when animals were

fed more frequently. For Exp.3, passage rates estimated via markers were similar to rates from evacuation when APL was used as the internal marker. Under most conditions, APL and IADF yielded similar passage rates via evacuation. Dry matter fill estimates from ruminal marker curves were significantly higher than those measured by evacuation. In conclusion, ruminal evacuation appears to be a viable approach for estimating particle passage, however, timing and number of evacuations should be considered.
(Key Words: Rate of Passage, Rumen Evacuation, Feeding Frequency, Markers.)